Occurrence of Donor Langerhans Cells in Mouse and Rat Chimeras and Their Replacement in Skin Grafts

Hong-Duo Chen, M.D., Chenglin Ma, M.D., Jing-Tao Yuan, Ya-Kun Wang, and Willys K. Silvers, Ph.D.
Department of Dermatology, No. 1 Hospital of China Medical University, Shenyang, People's Republic of China, and
Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Evidence is presented that some endogenous Langerhans cells (LCs) may persist indefinitely in skin grafts. This evidence is based on the observation that although 2 weeks after grafting F_1 hybrid mice and rats with genetically compatible skin, most of the LCs in the grafts were replaced with those of the host, some LCs of graft origin persisted for as long as the grafts were followed (154 days in mice and 249 days in rats). It has also been demonstrated that the spleen may be as good a source of LCs as the marrow. Thus, 6 weeks after lethally irradiated mice were restored with F_1 hybrid spleen cells, most of the LCs in the epidermis of their pinnae were of donor origin. LCs of donor origin also were found in the epidermis of the pinnae of animals that had been inoculated at birth with spleen and lymph node cells (mice) or bone marrow cells (rats). Hence the occurrence of these cells provides another means of confirming that tolerance (chimerism) has been induced. *J Invest Dermatol 86:630–633, 1986*

Although there is now convincing evidence that Langerhans cells (LCs) are antigen-presenting cells [1–3] that function in the skin and that, under normal conditions, they are the only cells of the mammalian epidermis that bear Ia antigens [4–6] and receptors for Fc-IgG [7], there are discrepancies concerning the life span of these cells. While Katz and his associates [8] found the LCs of some A/J skin grafts on (A/J × BALB/c)F_1 mice to be completely replaced with host LCs by 49 days posttransplantation, and Woodward et al [9] reported that in long-term (4 months) radiation chimeras, cells synthesizing Ia in the epidermis were exclusively of donor origin, the observations of Krueger et al [10,11] indicate that LCs are much more stable. Indeed, the data of these investigators, based on the persistence of LCs in human skin transplanted to nude mice, suggest that LCs or LC precursors are capable of dividing in the skin or, alternatively, represent an extremely long-lived cell population. Because of these conflicting reports we initiated experiments in both mice and rats that also were aimed at determining the persistence of donor LCs in skin grafts. Moreover, because it has been demonstrated that LCs have a bone marrow origin [8,12], we thought it conceivable that their precursors might occur in the spleen as well. Accordingly, we have evaluated the ability of donor spleen cells to repopulate the epidermal LC population of mice following lethal irradiation. We have also determined the frequency of donor LCs in the ear skin of rats and mice rendered tolerant at birth with either bone marrow cells (rats) or with a mixture of spleen and lymph node cells (mice). Our results indicate that some donor strain LCs may persist indefinitely in skin grafts, that the spleen may be as good a source of LCs as the marrow, and that the capacity of donor bone marrow and lymphoid cells to express themselves as LCs in the host provides an excellent means of confirming that tolerance (chimerism) has been induced.

Manuscript received October 3, 1985; accepted for publication January 22, 1986.
Supported by grants from the Chinese Academy of Sciences (B10-230) and from the National Institutes of Health (AM-34878 and CA-18640).
Reprint requests to: Hong-Duo Chen, M.D., Department of Dermatology, No. 1 Hospital of China Medical University, Shenyang, People's Republic of China.

Abbreviations:
A: A/Ss mice
B6: C57BL/6Ss mice
BN: BN/Ss rats
LC: Langerhans cell
Lew: Lewis/Ss rats
Lew.1N: Lewis.1N/Ss rats
MHC: major histocompatibility complex
PBS: phosphate-buffered saline, pH 7.3
Skn: skin-specific antigens

MATERIALS AND METHODS

Animals Major histocompatibility complex (MHC) incompatible strain A/Ss (A, H-2^a) and C57BL/6Ss (B6, H-2^b) mice and their F_1 hybrids, as well as Lew.1N/Ss and (Lew/Ss × BN/Ss)F_1 [hereafter (Lew × BN)F_1] hybrid rats were used. These substrains are maintained by Silvers at the University of Pennsylvania. Lew.1N and BN are MHC compatible (RT1^n) whereas Lew, which is congenic with Lew.1N, is RT1^l. Donor and recipients were sex-matched.

Cell Suspensions Bone marrow, spleen, and spleen and lymph node cell suspensions were prepared in Hanks' balanced salt solution according to procedures described elsewhere [13].

Chimerism Chimerism was induced in neonatal rats as well as in both neonatal and adult mice. It was induced in neonatal (less than 18 h old) Lew.1N rats by an i.v. injection of 50×10^6 viable (Lew × BN)F_1 hybrid bone marrow cells and in neonatal strain A and B6 mice by an i.v. injection of 20×10^6 (A × B6)F_1 lymph node and spleen cells immediately after the recipients had received 300 R (CO-60 γ source). The procedures for producing these tolerant animals have been described elsewhere [13]. Adult radiation chimeras were produced by inoculating strain A and B6 mice i.v. with 50×10^6 viable (A × B6)F_1 spleen cells immediately after they had received 900 R.

Skin Grafting Skin grafting was carried out according to procedures described elsewhere [14].

Anti-Ia Monoclonal Antibodies The mouse IgG monoclonal antibodies OX3 and OX4 were obtained from Sera-lab Ltd. (Sussex, England) and diluted appropriately (1:100 for OX3 and 1:400 for OX4) with phosphate-buffered saline, pH 7.3 (PBS), before

0022-202X/86/$03.50

use. OX4 binds to Ia antigens of all rats whereas OX3 binds to Ia antigens of Lew rats and B6 mice but not to BN or Lew.1N rats [15]. Preliminary experiments also indicated that OX4 reacts with the LCs of strain A but not of B6 mice. OX3 also reacts with the LCs of strain A mice but so weakly that it is not possible to enumerate LCs or even to identify typical LCs when this monoclonal antibody is employed in the indirect immunofluorescence staining procedure.

Ia Indirect Immunofluorescence Staining of LCs and Their Enumeration LC were identified with anti-Ia monoclonal antibodies OX3 and OX4, employing an indirect immunofluorescence procedure described elsewhere [16]. Briefly, the procedure entailed separating with watchmaker's forceps the epidermis from the dermis of the piece of skin to be analyzed after it had been incubated for 2 h in a phosphate-buffered EDTA solution; soaking the epidermal sheets in acetone for 20 min at room temperature; cutting the epidermal sheets into 2 pieces (each about 4 × 4 mm) and immersing one for 16 h at 4°C in OX3 and the other, under the same conditions, in OX4; and incubating both sheets of epidermis for 1 h at 37°C with fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of goat antimouse IgG (catalog no. 1311-3291, Cappel Laboratories, Cochranville, Pennsylvania) diluted 1:60 with PBS.

The enumeration of these cells has also been described previously [17].

RESULTS

Persistence of Donor LCs in Skin Grafts To determine how long the endogenous population of LCs may persist in a skin graft, strain A or B6 ear or trunk skin grafts that had been in residence on (A × B6)F_1 mice for various periods, as well as Lew.1N trunk skin grafts that had been accepted for similar periods on (Lew × BN)F_1 rats, were removed (either partly or entirely) and stained with OX3 and OX4 as noted above. The results (Table I) indicate that although there is a rapid replacement of graft LCs with those of the host, such that by 2 weeks after grafting an average of 60–80% of the LCs in the grafts have been replaced with those of host origin, some donor LCs persist for long periods. In fact, not only were some donor LCs present in grafts that had been accepted by mice for 154 days and by rats for 249 days but, in general, the average percentage of donor LCs seemed to persist at around 20% regardless of how long the grafts had been on.

Spleen As a Source of Epidermal LCs Our assessment of the spleen as a source of LCs involved reconstituting lethally irradiated strain A and B6 mice with (A × B6)F_1 spleen cells and periodically assaying these animals for donor LCs by removing a portion of their pinna and staining its epidermal sheet with OX3 and OX4, respectively. The results (Tables II, III) indicate that the spleen may be as good a source of LCs as the marrow. Thus, although donor LCs were not observed in the ear epidermis of the recipients until the 4th week after they were reconstituted, by 6 weeks about 80% of all the LCs in the ear were of donor origin. Nevertheless, even 30 weeks after spleen cell reconstitution a significant number of host LCs were still detected in the epidermis.

Donor LCs in Mice and Rats Rendered Immunologically Tolerant at Birth In addition to determining the proportion of donor LCs in the epidermis of the ear of lethally irradiated and spleen cell-reconstituted mice, we also determined the frequency of these cells in the ear epidermis of adult Lew.1N rats that had been rendered tolerant at birth with 50×10^6 (Lew × BN)F_1 bone marrow cells, and of adult strain A and B6 mice that had been inoculated at birth with 20×10^6 (A × B6)F_1 spleen and lymph node cells (following sublethal irradiation). In these experiments all recipients were challenged with an appropriate skin

Table I. Percentages of Host LCs in Mouse (Strain A and B6) and Rat (Lew.1N) Skin Grafts after Various Periods of Time Following Transplantation to (A × B6) F_1 and (Lew × BN) F_1 Hybrids, Respectively

Skin Graft	Day Tested	No. Tested	Host LCs/Total LCs[a] Range (%)	Host LCs/Total LCs[a] Average (%)
A	15	10	29–91	63
	28	10	36–92	74
	154	10	65–100	79
B6	15	8	53–94	80
	27	8	65–93	82
	154	7	39–89	72
Lew.1N	13–16	10	35–80	60
	49	10	52–91	78
	100–106	10	50–96	75
	151	9	35–90	75
	192–249	8	67–115	84

[a]These percentages are based upon the appropriate proportions of $OX3^+$ and $OX4^+$ LCs.

Table II. Langerhans Cells[a] of Donor Origin in the Ear Epidermis of Strain A Mice Inoculated i.v. with (A × B6)F_1 or Syngeneic Spleen Cells after Lethal Irradiation

Spleen Cell Donor	Week[b] Tested	$OX3^+$ LCs[c]/mm²	$OX4^+$ LCs[d]/mm²	Donor Cells/Total Cells (%)
(A × B6)F_1	2	0[e]	680	0
	4	80	560	14
	5	280	736	22
	6	680	765	89
	7	634	893	71
	30	688	968	71
A	4	0	780	0
	5	0	656	0
	6	0	768	0
	7	0	852	0
	30	0	992	0

[a]Each result represents the mean of 6 animals.
[b]After irradiation and reconstitution.
[c]Representing LCs of (A × B6)F_1 origin.
[d]Representing LCs of A and (A × B6)F_1 origins.
[e]Although OX3 reacts with strain A LCs, the reaction is so weak that entire cells are not discernible. Hence, only cells that were entirely visible were recorded as positive.

Table III. Langerhans Cells[a] of Donor Origin in the Ear Epidermis of B6 Mice Inoculated i.v. with (A × B6)F_1 or Syngeneic Spleen Cells after Lethal Irradiation

Spleen Cell Donor	Week[b] Tested	$OX4^+$ LCs[c]/mm²	$OX3^+$ LCs[d]/mm²	Donor Cells/Total Cells (%)
(A × B6)F_1	2	0	304	0
	3	0	260	0
	4	72	428	17
	6	292	346	84
	7	292	312	92
	15	232	336	69
	30	356	480	74
B6	2	0	264	0
	3	0	288	0
	4	0	292	0
	5	0	423	0
	6	0	424	0
	15	0	370	0
	30	0	523	0

[a]Each result represents the mean of 6 animals.
[b]After irradiation and reconstitution.
[c]Representing LCs of (A × B6)F_1 origin.
[d]Representing LCs of B6 and (A × B6)F_1 origins.

Table IV. Occurrence of Donor Strain LCs in the Ear Epidermis of Rats (Lew.1N) and Mice (strain A and B6) Rendered Tolerant at Birth[a]

Chimera	No. Tested	Graft	Graft Survivals (days)	Donor LCs/Total LCs[b] Range (%)	Donor LCs/Total LCs[b] Average (%)
Lew.1N/(Lew × BN)F_1	11	BN	11 × >200	20–53	32
A/(A × B6)F_1	10	B6	10 × >200	9–50	23
A/(A × B6)F_1	10	B6	17,18,20,39, 50,72,84, 107,182,183	23–45	33
B6/(A × B6)F_1	12	A	12 × >200	25–50	41
B6/(A × B6)F_1	7	A	15,34,57,68, 81,83,119	27–50	42

[a]The Lew.1N rats were injected at birth with 50 × 10^6 (Lew × BN)F_1 bone marrow cells and the strain A and B6 mice were injected at birth with 20 × 10^6 (A × B6)F_1 spleen and lymph node cells (after 300 R irradiation). Animals were skin grafted at 2 months of age and assayed for the occurrence of donor LCs 150 days later.

[b]These percentages are based upon the appropriate proportions of $OX3^+$ and $OX4^+$ LCs.

graft (in rats prepared from adult BN animals and in mice from strain A or B6 neonatal trunk skin) at 2 months of age and, regardless of the fate of these grafts, the recipients were assessed for the occurrence of donor LCs 150 days later. The results (Table IV) indicate that, regardless of whether the test skin graft was accepted or not, LCs of donor (F_1 hybrid) origin were always detected in the ear skin of the host. Moreover, the percentages of donor cells, which displayed similar variations in graft accepters and rejecters, ranged from 9–53% with no apparent species or strain differences.

DISCUSSION

Although we found that the LCs of skin grafts seem to be rapidly replaced with those of the host, an observation in accord with the observation of Katz et al [8] who reported that 11 days after transplantation 61% of the LCs of A strain skin grafts on (A × BALB/c)F_1 hosts were of host origin, in contrast to their findings, we found some native LCs to persist for very long periods. Thus, even after Lew.1N grafts had been accepted for 192–249 days by (Lew × BN)F_1 hosts about 16% of their LCs remained of donor origin. Our results are therefore more in accord with those of Streilein et al [18] who, on the basis of the rejection of retransplanted Ia-disparate murine skin grafts, also suggested that LCs may not be completely replaced in long-tolerated grafts. They also are consistent with the observations of Krueger et al [10,11] who concluded that human LCs are either long-lived (cannot be dislodged) or can proliferate locally. Indeed, with regard to the latter possibility, it has recently been reported that human LCs can be found in various cell cycle phases, indicating that they are able to proliferate in situ [19]. On the other hand, it is also possible that there are 2 subpopulations of Ia-positive dendritic cells in the epidermis, a mobile one and a self-perpetuating and relatively fixed one. Moreover, these 2 populations may not only be quite distinct but they may serve different functions.

We have confirmed the recent report of Roberts et al [20] that there are LC precursors in the spleen of adult mice. In fact, the observation that the numbers of donor LCs in the ear skin of adult mice that had been inoculated at birth with spleen and lymph node cells were similar to chimeric populations in the ear skin of rats rendered tolerant at birth with bone marrow cells (see Table IV), also indicates that the spleen is as good a source of LCs as the marrow. And this occurred despite the fact that while most of the rats were permanently tolerant of skin allografts, a considerable number of the chimeric mice were not. Nevertheless, this is not surprising since, unlike the situation in animals rendered tolerant with bone marrow cells, those rendered tolerant with spleen and lymph node cells are still able to react against skin-specific (Skn) antigens [21]. In mice these Skn antigens are much more likely to provoke graft rejection in adult than in neonatal skin [22] and that is why neonatal skin grafts were employed in this study. Had adult skin grafts been employed it is likely that all of them would have been rejected, at least when B6 animals were the recipients [22].

The capacity of donor bone marrow and spleen cells to express themselves as LCs in the host provides another method for confirming that tolerance (chimerism) has been induced when the appropriate class II antigenic differences are involved. This method is especially attractive since all it requires is removing a small piece of the pinna. In a previous study [23] a cytotoxic test on spleen cell suspensions was employed to demonstrate chimerism in $H\text{-}2^a$ and $H\text{-}2^b$ mice that had also been inoculated at birth with 20 × 10^6 F_1 hybrid spleen and lymph node cells. Although that assay revealed levels (2–34%) of chimerism comparable to those (9–53%) reported here, 2 mice that permanently accepted their test grafts gave no indication that their spleen possessed donor lymphoid cells.

REFERENCES

1. Streilein JW, Bergstresser PR: Ia antigens and epidermal Langerhans cells. Transplantation 30:319–323, 1980
2. Ptak W, Rozycka D, Askenase PW, Gershon RK: Role of antigen-presenting cells in the development of contact hypersensitivity. J Exp Med 151:362–375, 1980
3. Steinman RM: Dendritic cells. Transplantation 31:151–155, 1981
4. Rowden G, Lewis MG, Sullivan AK: Ia antigen expression on human epidermal Langerhans cells. Nature 268:247–248, 1977
5. Klareskog L, Tjernlund U, Fursum U, Peterson PA: Epidermal Langerhans cells express Ia antigens. Nature 268:248–250, 1977
6. Stingl G, Katz SI, Shevach EM, Wolff-Schreiner E, Green I: Detection of Ia antigens on Langerhans cells in guinea pig skin. J Immunol 120:570–578, 1978
7. Stingl G, Wolff-Schreiner EC, Pichler WJ, Gschnait F, Knapp F, Wolff K: Epidermal Langerhans cells bear Fc and C3 receptors. Nature 268:245–246, 1977
8. Katz SI, Tamaki K, Sachs DH: Epidermal Langerhans cells are derived from cells originating in bone marrow: Nature 282:324–326, 1979
9. Woodward JG, Shigekawa BL, Frelinger JA: Bone marrow-derived cells are responsible for stimulating I region-incompatible skin graft rejection. Transplantation 33:254–259, 1982
10. Krueger GG, Daynes RA, Emam M: Biology of Langerhans cells: selective migration of Langerhans cells into allogeneic and xenogeneic grafts on nude mice. Proc Natl Acad Sci USA 80:1650–1654, 1983
11. Krueger GG, Emam M: Biology of Langerhans cells: analysis by experiments to deplete Langerhans cells from human skin. J Invest Dermatol 82:613–617, 1984
12. Frelinger JG, Hood L, Hill S, Frelinger JA. Mouse epidermal Ia molecules have a bone marrow origin. Nature 282:321–323, 1979
13. Billingham RE: The induction of tolerance of homologous tissue grafts, Transplantation of Tissues and Cells. Edited by RE Billingham, WK Silvers. Philadelphia, Wistar Institute Press, 1961, pp 87–104
14. Billingham RE: Free skin grafting in mammals, Transplantation of Tissues and Cells. Edited by RE Billingham, WK Silvers. Philadelphia, Wistar Institute Press, 1961, pp 1–24
15. McMaster WR, Williams AF: Identification of Ia glycoproteins in rat thymus and purification from rat spleen. Eur J Immunol 9:426–433, 1979
16. Nordlund JJ, Ackles A: A method of quantifying Langerhans cells in epidermal sheets of human and murine skin. Tissue Antigens 17:217–225, 1981
17. Chen HD, Yuan J, Wang Y, Silvers WK: Distribution of ATPase-positive Langerhans cells in normal adult human skin. Br J Dermatol 113:707–711, 1985

18. Streilein JW, Strome PG, Gruchalla RS, Wood PJ: Analysis of neonatally induced tolerance of H-2 alloantigens. Transplantation 35:474–478, 1983

19. Czernielewski J, Vaigot P, Prunieras M: Epidermal Langerhans cells—a cycling population. J Invest Dermatol 84:424–426, 1985

20. Roberts LK, Krueger GG, Daynes RA: Correlation between the inducible keratinocyte expression of Ia and the movement of Langerhans cells into the epidermis. J Immunol 134:3781–3784, 1985

21. Desquenne-Clark L, Kimura H, Silvers WK: Evidence that major histocompatibility complex restriction of foreign transplantation antigens occurs when tolerance is induced in neonatal mice and rats. Proc Natl Acad Sci USA 82:6265–6267, 1985

22. Silvers WK, Wachtel SS, Poole TW: The behavior of skin grafts incompatible with respect to skin alloantigens on mice rendered tolerant at birth with lymphoid cells. J Exp Med 143:1317–1326, 1976

23. Fleming HL, Silvers WK: An immunogenetic analysis of Skn antigens in mice. Immunogenetics 14:517–526, 1981